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This Issue:

Primates in Research
Endotoxic Shock Therapy
Detecting Carriers of DMD
Genbank

By
Greg Freiherr

Laser Surgery Mends Nerves

A laser beam coagulates red blood cells encircling the ends of a severed nerve, forming an adherent sleeve of tissue that holds the two ends together. Within this sleeve, new nerve fibers from the ends grow together.

Whether laser repair of the damaged nerve will produce better results than suturing the nerve ends together is not known. The technique has just recently been applied to humans, and conclusions on the return of the nerve function cannot yet be drawn. Only white rats, monkeys, and baboons have been completely evaluated.

The animals were studied at the University of Washington (UW) in Seattle, where Dr. Edward E. Almquist, a hand surgeon who developed the laser procedure, is a clinical professor in the orthopedics department of the medical school. Tests on the nonhuman primates were conducted at the UW Regional Primate Research Center in Seattle.

Severed rat nerve 8 weeks after repair with a laser: *coagulated red blood cells (nodules in center) still adhere to the healed junction.*

LASER *continued*

Success in the animal tests was judged mostly on the appearance of the repair sites several months after surgery. Nerves were examined with scanning transmission electron microscopy and light microscopy. "The external surface of the repair sites could often barely be distinguished from the adjacent nerve surface or from nerves that had not been severed," Dr. Almquist says. The quantitative evaluation of sensory return, however, could not be determined. "Injury to one nerve and the consequent loss of sensation affect coordination and dexterity but not necessarily gross motion," he says.

These questions will be decided through ongoing clinical studies at the Seattle clinic of Dr. Almquist and his colleagues in the Seattle Hand Surgery Group. They are applying the laser technique to people with nerve damage in the upper extremities.

Dr. Edward E. Almquist, Seattle hand surgeon and developer of the laser procedure for repairing nerves: *"If the (nerve) fibers can be closely aligned there is a much greater probability that significant sensation will return."*

Glass, sheet metal, and power tools inflict the majority of these injuries. According to a 1977 survey by the National Center for Health Statistics, more than 20,000 injuries to nerves in the arm, wrist, and hand occur annually. "Damage of this type can stop the means of employment for someone who requires a great deal of dexterity—someone like a watch repairman or a surgeon," Dr. Almquist says.

The injury is at least an inconvenience—a nagging, frustrating one that makes simple actions difficult. Writing, buttoning shirts, or just tying shoes can be a problem. The hope that surgical reconnection of the nerve might restore at least some sensation has been drawing people for years to the clinic of the Seattle Hand Surgery Group.

Before the laser was introduced, nerves were usually reconnected by suturing the severed ends together. This method is still generally used. It works, but it has some drawbacks. The fibers from each nerve end branch unpredictably, a phenomenon demonstrated in numerous animal experiments. Even the most advanced microsurgery, with sutures so small that individual nerve branches are tied together, cannot prevent these branches from sprouting outside the intended junction sites.

As a result, most nerve fibers do not reconnect with sister fibers from the other part of the nerve. Those that do reconnect usually do so incorrectly. "The fibers that normally provide feeling to the thumb might attach to fibers that serve the ring finger," Dr. Almquist says.

Children typically improve more than adults when severed nerves are sewn back together. The reason, according to Dr. Almquist, is that the child's brain is more adaptable. "Even though the nerve ends don't match very well, the brain is eventually able to make sense of the information it receives," he says.

There is another drawback to sewing nerves together. "The suture goes through the body of the nerve, so it does some damage," Dr. Almquist says.

To increase the number of nerve fibers that correctly reconnect and to reduce damage to the nerve, a variety of sleeves made of fibrinogen, silicon, glass, veins, or hypoallergenic collagen were developed and tested. None proved clinically practical until Dr. Almquist and his colleagues fashioned a natural sleeve from

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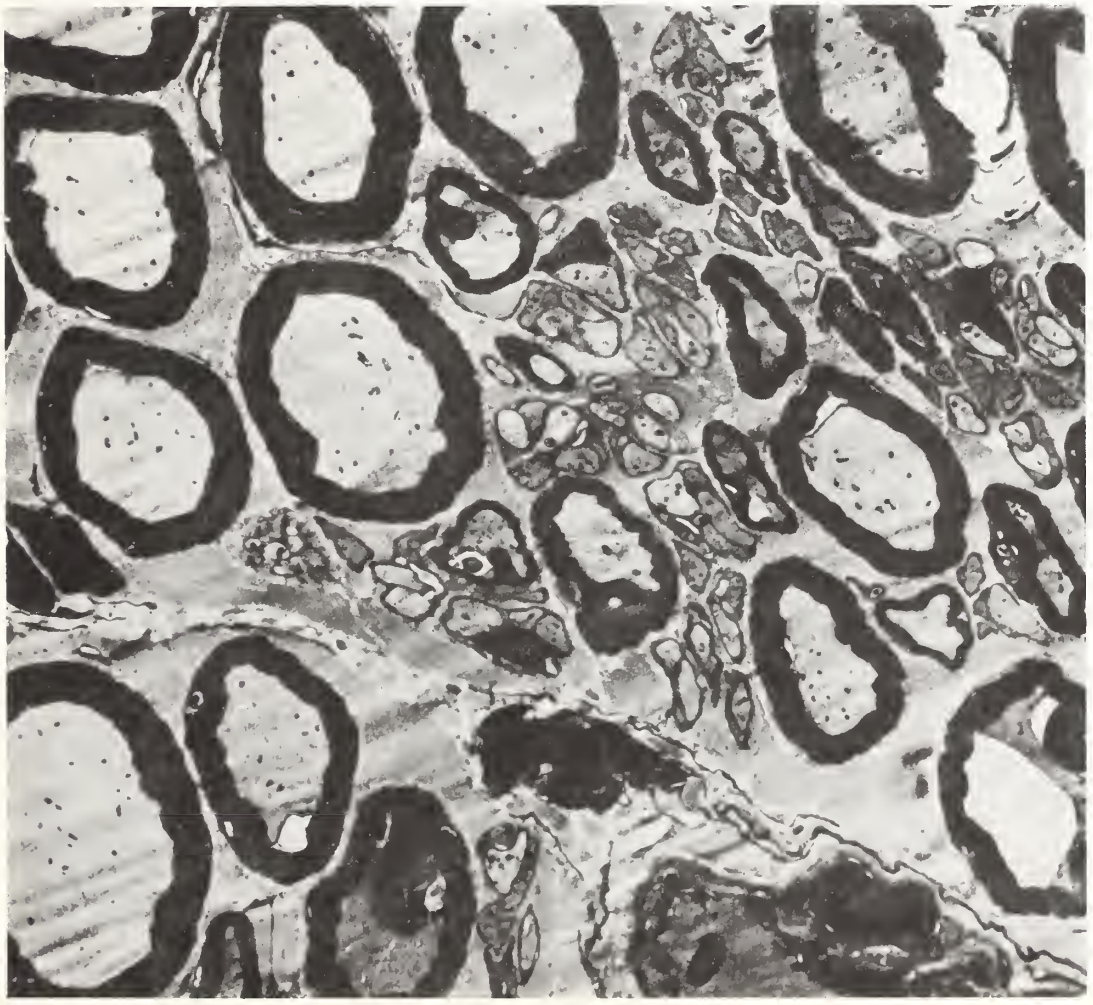
Two baboons at the Primate Field Station near Spokane, Washington: *aiding in the repair of nerves with laser light.*



Nerve fascicles (circles): *bundles of nerve fibers analogous to the wires inside an electrical cable.*

coagulated red blood cells. The sleeve directs the growing fibers to each other. It also holds nerve fibers together without sutures.

"Previously we didn't bother matching the nerve fibers because the branches would grow out of alignment and the effort would be wasted. But if the fibers can be closely aligned there is a much greater probability that significant sensation will return," he says.

When splicing a severed nerve with the laser, Dr. Almquist matches from each nerve end bundles of nerve fibers called fascicles. The process is analogous to matching and splicing the wires inside an electrical cable. Matching nerve fibers, however, is immensely more difficult than the task facing an electrician. Each fascicle measures about 0.1 millimeter in diameter and contains about 10,000 fibers.

The laser surgeon examines the nerve through a binocular microscope suspended over the patient. He looks on the surface of the fascicles for characteristic ring patterns; for example, a wide ring followed by two thin ones. Corresponding nerve bundles are held together with a forceps. Blood is applied around the junction site and the area is flashed with the laser.

The surgical system consists of an argon ion laser fitted with an optical fiber, a shutter, timer, and foot switch. Depressing the foot switch opens the shutter for a preset time. The laser beam travels through an optical fiber consisting of fired quartz. Its length is 4.5 meters; its core diameter is 400 microns, which is equivalent to the diameter of the graphite in a pencil.

The laser delivers only 0.75 watts of power, and its burst of light lasts just half a second. The blue-green light is absorbed by red-colored materials—the red blood cells. It is reflected by white materials—the nerves. As planned, red blood cells applied over

LASER *continued*

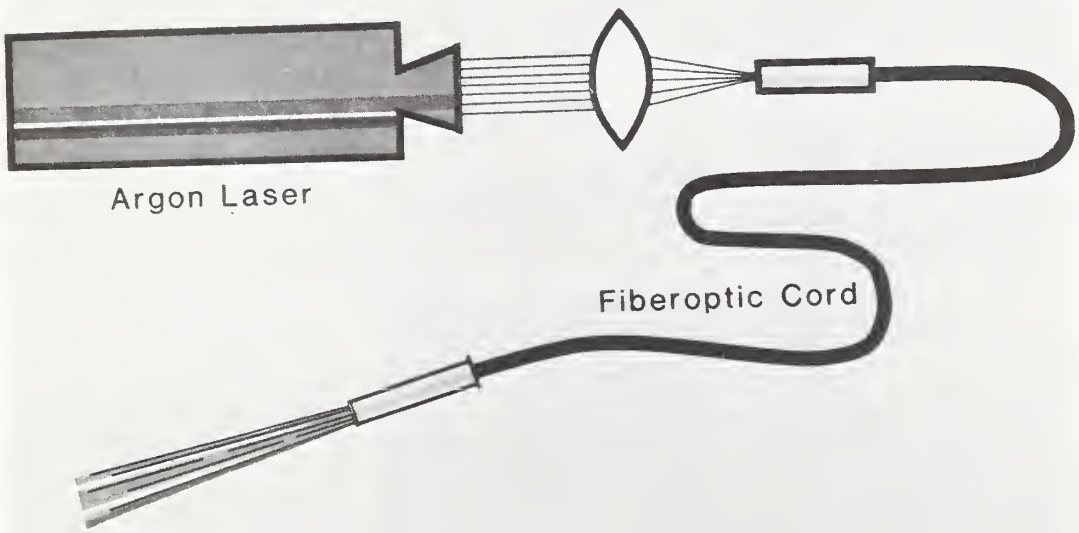
the nerve absorb the energy from the beam and form a sleeve of coagulated tissue. The white nerve fibers reflect the laser energy.

Although the laser light itself does not affect the nerve, heat from the coagulation of red blood cells might do some damage. Such a small quantity of heat is generated, however, that a means of measuring it has not yet been devised.

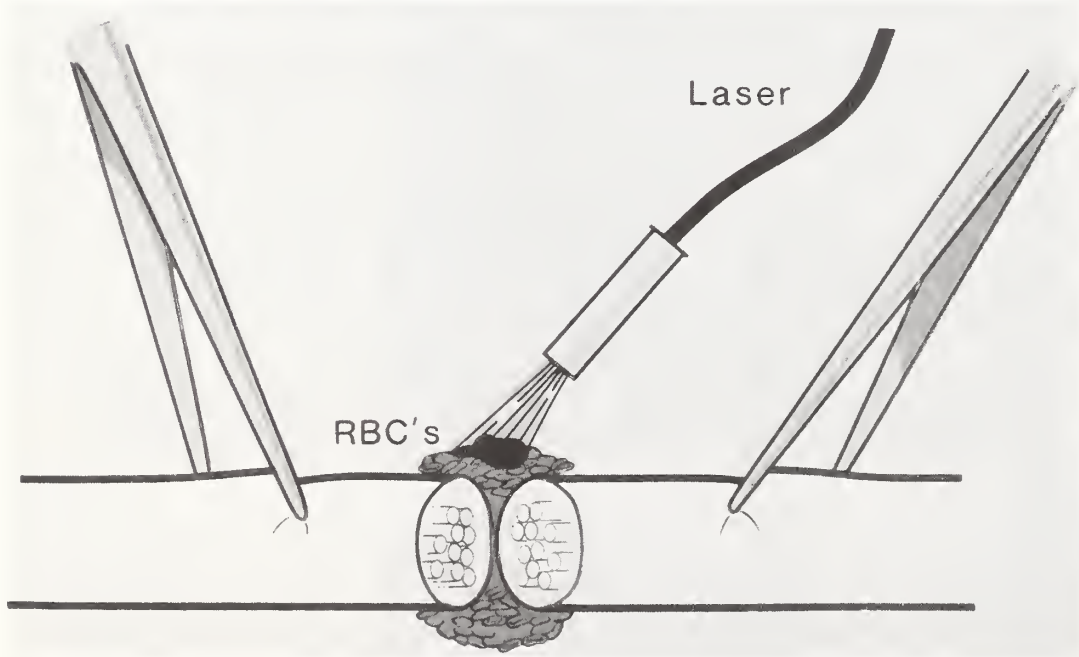
Feasibility of the technique was proved on rats in 1976, using an argon laser focused through a microscope. A prism and mirror assembly directed the beam. Although nerve repair appeared to be technically possible, the system was awkward and could not be used successfully on even a major nerve. When the microscope was replaced by an optic fiber system, developed by Dr. David Auth of the UW Seattle electrical engineering department, the technique became practical.

Tests on 32 rats confirmed that the procedure allowed severed nerves to heal. Electron microscopy and standard light microscopy of nerves harvested at various times following surgery showed how the healing progressed.

At first, adherent round globules formed from the red blood cells hold the nerve ends together. Within several days, collagen fibrils intermesh in the sleeve and replace the globules, and nerve fibers begin to sprout from the nerve ends. The sleeve of coagulated red blood cells constrains the



Surgical laser system



Laser light coagulates red blood cells (RBC's) encircling the severed nerve ends.

sprouts to the repair site. Within 3 months after surgery the repair site is nearly indistinguishable from the rest of the nerve surface as seen by electron and light microscopy.

In the rat, the laser-repaired nerve produced a return of muscle function. "A word of caution about interpreting results in rats: previous studies of other repair procedures including simple suturing have shown similar repair and a return of function," Dr. Almquist says.

The truly encouraging news came in the next phase of the research—the testing of 10 nonhuman primates at the UW Regional Primate Research Center in Seattle. Each animal was anesthetized. The left median nerve, which serves muscles of the forearm, thumb, elbow, and many joints of the hand, was cut and dissected microscopically into 3 or 4 fascicles. Blood mixed with heparin was applied over the fascicles as they were held together with forceps. A flash of laser light coagulated the blood, creating a sleeve over the two nerve ends.

The ulnar nerve, which serves many of the same areas as the median nerve, was used as a control. It was exposed, covered with the blood-heparin mixture, and flashed with the laser. Areas of the median and ulnar nerves exposed to the laser were retrieved 4 to 6 weeks after surgery.

As in the rats, primate nerve repair sites were nearly indistinguishable from other surface areas. Nerve fibers were channeled and joined together. Few fibers escaped from the sleeve. Dr. Almquist judged the alignment and density of the regrown nerve ends in the primate to be "very satisfactory." There was no excessive scarring. The ulnar nerve, which was not severed, showed no damage due to exposure to laser light or to heat resulting from the coagulation of blood cells.

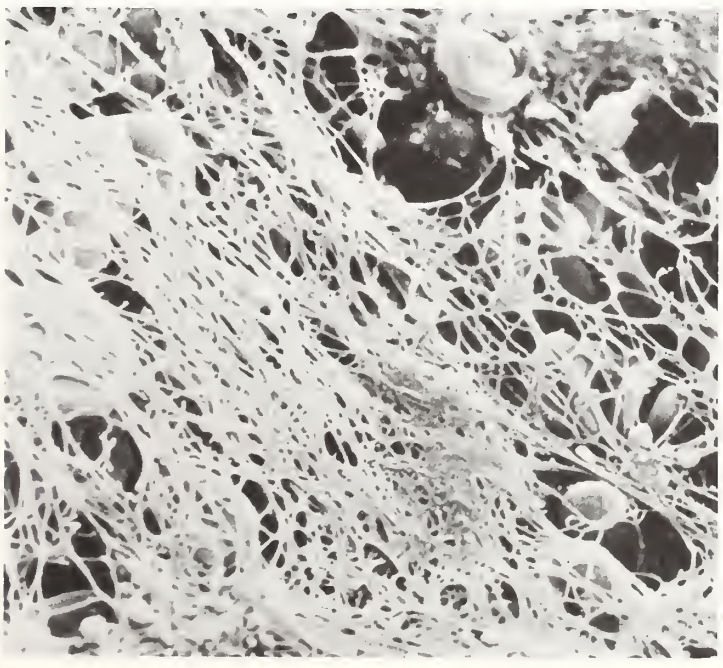
But the ultimate question is whether the repair of nerves with a laser will return more sensation to fingers and hands than does conventional surgery in which the nerve ends are sewn together. The clinical studies now going on should prove the answer. Evaluating the return of sensation, however, will be subjective.

"You can't say that a patient has a 50 percent return of feeling, because there is no good way to quantitate feeling. What's normal feeling? What's half normal feeling? We don't know," Dr. Almquist says. "We will have to depend on comparisons by the patient of sensation after surgery to sensation before and after the accident."

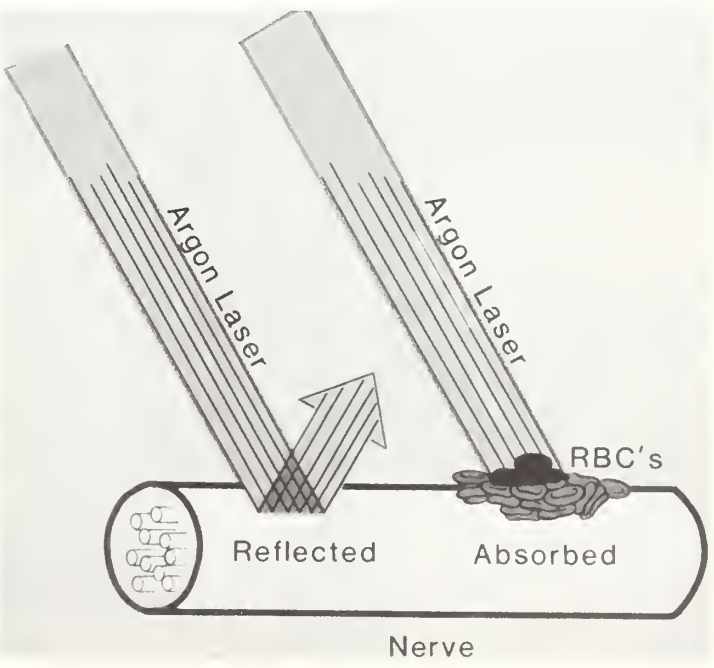
Preliminary results will soon be available. Sensation begins to return about 3 to 4 months after surgery, and it continues to increase for another year. "But we will have to wait years for the final answer—until enough time has elapsed and enough patients have undergone the treatment," says Dr. Almquist, "that we can confidently render a judgment." R

The University of Washington Regional Primate Research Center in Seattle is supported by the Animal Resources Program of the NIH Division of Research Resources.

Coagulated tissue surrounding the severed nerve ends: *a natural sleeve that holds the ends in place and guides growing nerve fibers to their targets.*



Red blood cells (RBC's) absorb the blue-green laser light, but white nerve fibers reflect it: *applying the laser's power while protecting the nerve.*



Primates in Biomedical Study: Resources To Be Conserved

At the Regional Primate Research Centers (PRC's), the animal—not the facility—is the resource. Living animals are the proving grounds of ideas that cannot be tested on people. They must be in excellent health to ensure that test data are valid. When the animals die, tissues and specimens are conserved for study. About 13,000 primates, representing 44 species, are now housed in the Regional PRC's, which are supported by the Animal Resources Program of the NIH Division of Research Resources (DRR). The large number and variety of animals are distinctive of PRC's.

Core breeding programs supply about 1,800 animals per year—about 70 percent of the subjects needed for center research projects. The University of Washington PRC maintains a breeding colony near Spokane, Washington, and a small research station in Borneo, Indonesia. The breeding colony, with some 1,500 primates, supplies animals to the PRC laboratories in Seattle, where studies in neurophysiology, cardiovascular physiology, developmental biology, metabolism, and genetics are conducted. Some research is also done at the breeding station, particularly long-term studies of aging. The research station in Borneo serves as an outpost for the study of primates in the wild, where information useful in care, handling, and breeding of the animals can be obtained.

In an interview with Greg Freiherr of the *Research Resources Reporter,* the director of the University of Washington PRC, Dr. Orville Smith, discussed the role of animals in biomedical research, ethical considerations in their use, and alternatives to animal studies.

Dr. Orville Smith, director of the University of Washington Regional Primate Research Center in Seattle: *"People shouldn't be the first living organisms to take an experimental drug or vaccine."*

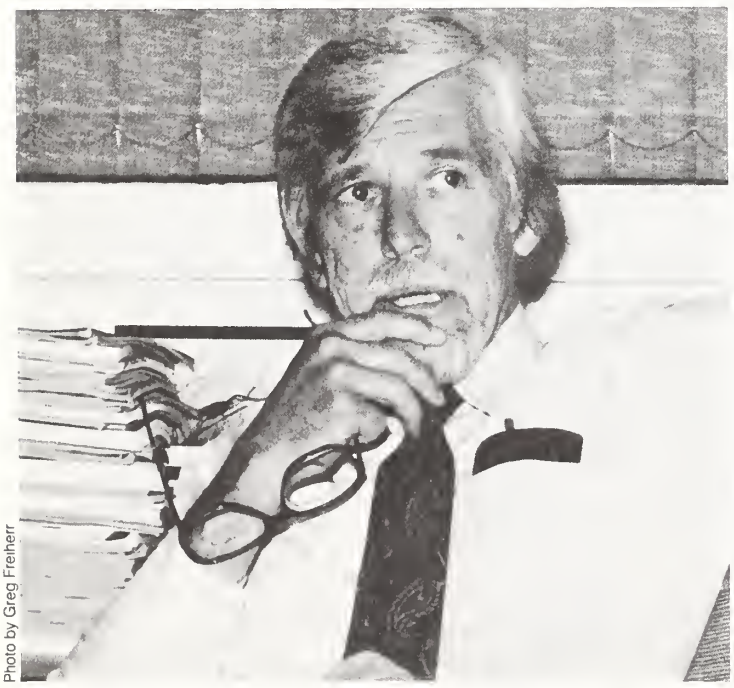


Photo by Greg Freiherr

Why use nonhuman primates in research?

In many cases they provide research opportunities that no other kind of experimental animal can provide. Humans and nonhuman primates have the same essential characteristics with regard to anatomy and physiology. Our biochemical makeup is similar. From a biological standpoint, the probability is much higher that research carried out on a nonhuman primate is going to be directly applicable to human beings than research using some other animal.

What types of studies are done best on primates?

The visual systems of the monkey and the human are almost indistinguishable, whereas the visual systems of the cat, dog, guinea pig, and rabbit are all very different from man's. The basic organization of the human and nonhuman primate brains is essentially the same: humans have a central sulcus, a motor cortex, and a sensory cortex in the same places as does the nonhuman primate. Because of the similarities in brain structure there is reason to believe that the neural mechanisms underlying the animal's behavior are similar to the human's. Another example is the cardiovascular system. The baboon is a better model of blood flow to the kidney during heavy exercise than is the dog. One reason may be that the dog is a running animal with a large spleen, which provides a reservior of blood. The dog acquires extra oxygen-carrying capacity in the bloodstream during exercise because the spleen constricts, releasing extra blood. The baboon and the human don't have this capacity.

Then animals are chosen according to the goals of the specific study?

Exactly. It depends on the mechanism we want to study. We'd much prefer that animals other than the primate be used whenev-

er possible. I really believe use of the primate should be restricted to studies in which it's obvious that the primate must be the model.

This is mostly because primates are expensive, isn't it?

No. Not entirely. Economics enter into the decision, but the major factor is conservation—the general principle of using our animals efficiently and wisely.

Isn't it also true that the use of animals phylogenetically closer to man generates greater concern among animal rights activists than the use of animals farther down the evolutionary ladder?

I think that's undoubtedly a true statement, but there is much concern today about the welfare of all animals in research. It's an issue that is being hotly debated. Historically, and even today, the public's greatest concern has been with animals that are also kept as pets, such as cats and dogs.

The central point in this debate is whether animals are really needed to conduct research. Are they really needed?

At this time they are absolutely indispensable. People shouldn't be the first living organisms to take an experimental drug or vaccine. And even after the tests are completed, we need animals to ensure the product's quality. Some years ago, quality control failed at a company that makes polio vaccine, and a number of children contracted the disease from the vaccine. If no animals were ever used for testing, our polio vaccination program could be worse than the natural occurrence of the disease. The need for animals in research is simply one aspect of the critical and continuous need for animals in our present system of health care. You either settle for unnecessary human suffering and death or you use animals.

The human-animal link: *"Our best protection against cruelty to animals is the people who handle the animals."*

Do you agree that alternatives such as in vitro testing should be used to reduce the use of animals?

A lot of preliminary research is already being done in vitro. That's great. The more we can do in cell culture, the better we like it. But there are limitations. We can go only so far. With in vitro methods, we aren't going to learn a single thing about how the integrated brain operates, for example. We need a functioning, intact brain—an organ with billions of nerve cells interacting with each other.

In the areas suited to alternative methods, could these alternatives be used more extensively?

In your question you presume that the alternative technology is available and that maybe there isn't the interest to apply it. That's not the case. Cell culture is not that old. Investigators are and have been applying it wherever it can be applied. Work involving recombinant DNA is being done now because the tools are available. We're developing alternatives by our own volition, and we're applying them when the technology fits the problem. What's exciting is that the technology is becoming available, and researchers are now starting to use it.

But isn't there some resistance in the biomedical community to using the alternatives that are now available and applicable?

I don't think so. While the amount and quality of biomedical research has been increasing in the last 15 years, the number of animals used has decreased by 35 percent. Most of us, I think the great majority, would prefer to use alternatives, but they simply don't exist right now for many major avenues of research. Existing alternatives are being used. Ironically, some of the ostensible alternatives beg the question of whether it is morally right to use animals. One example is the use of tissue cultures. The cells for tissue cultures have to come from animals, and even after they're extracted, the cells are alive. So where do you make the cutoff?

Isn't the cutoff determined by either of two attitudes? One relates to ethics: Should animals be used? The other is concerned with the kind of experiments using animals—the cruelty question.

Those who cite the ethics of animal research state that no form of animal life should be injured for any reason. Some adhere very conscientiously to their code of ethics. They are vegetarians. They do not wear leather shoes, and, similarly, they are very careful not to wear clothes made of leather or fur. I don't knock that. I admire ethical principles. If a person believes in them and acts accordingly, I respect that. But I just don't see how any person who has a real ethical sensibility can say that his or her children, friends, or parents should suffer or die rather than take the life of an animal.

INTERVIEW *continued*

Is the antivivisection movement composed mostly of absolutists?

There are all varieties of people in the movement. Some are absolutists. But many want to work with the scientists to improve the daily existence of the animal in the laboratory. They're not saying: "Stop your research." They're saying: "Let's make sure that these animals are cared for in the best possible fashion, that they experience the least distress possible, and that they have the best facilities and the best veterinary care."

Now you're talking about people who oppose cruelty to animals. Why aren't they satisfied with existing guidelines for the care and handling of animals?

Regulations specified in the Animal Welfare Act and administered by the U.S. Department of Agriculture address the environmental conditions under which research animals are kept—cage size, lighting, ventilation. That sort of thing. Currently the major issue is whether new laws should be passed to deal with how animals are used in experiments.

Are there no existing guidelines for how the animals are used?

Certainly there are guidelines. The NIH Guide deals with some of the basic issues regarding analgesia, anesthesia, postsurgical care, and acceptable methods of euthanasia. Scientists working at the primate center have for years adhered to more detailed guidelines developed by their professional societies, including the American Physiological Society, the National Society for Medical Research, and the American Veterinary Medical Association. These guidelines are specifically intended to minimize pain that might be experienced by experimental animals. The issue now has more to do with regulations and laws. Some believe that the Congress and the Federal agencies should play a more directive role in defining, regulating, and enforcing such guidelines.

Isn't that role now being played primarily by the university?

At our primate center, we rely on a university-wide Animal Care Committee to review the protocols of all grant requests to assure that animals are used properly. It is composed of veterinarians, researchers, administrators, and a member of the lay public. On a day-to-day basis, our veterinarians and their staffs monitor the condition of all animals. They will stop any ongoing experiment that they believe is not adhering to the principles of good care and ethical treatment. Unnecessary pain is one criterion they are concerned about.

How do you define "unnecessary pain?"

In most animal research, pain is not a common feature. Unnecessary pain is pain inflicted without a reason. We are opposed to this.

What happens if the veterinarian stops an experiment?

He ordinarily recommends alternative approaches so that the research can continue. There are often different ways to approach a research problem, and a scientist must do what he's been trained to do—what seems to be the most logical approach to answering an important question. If in pursuing that goal, the animal will have unnecessary pain, the research has to be done another way, or it has to stop.

Some researchers reportedly have discontinued their work in recent years because they didn't believe the research goals justified the pain and injury experienced by the animals. Have you or your staff ever felt that you're going beyond what should be done?

I hope that no one here would knowingly go beyond what he should do. But there is, and I think there always will be, the feeling that the conditions and treatment of animals can be better. We constantly try to improve the quality of animal care. As an example, we have found ways to drastically reduce our need for the restraint chair. Its use has made possible a tremendous amount of research that required long-term measurement of physiological or neurological functions. But we don't like the idea of an animal sitting in a chair for several weeks at a time. So, we have set out to reduce as much as possible our use of the restraint chair.

We did so by developing new techniques for surgical implants, by using a tether system for fluid lines that allows the animal freedom of movement, and by using vests that carry radio transmitters. Now we're developing a cage that turns into a temporary chair. When it's time to run tests, the animal hops onto the seat and sticks his head through the top of the cage. We give him a little piece of apple and wheel the whole cage into the study room. When the testing is done, the seat is removed and the animal is back in his cage.

Why have you made these changes?

They are ways of making the research easier on the animal. It wasn't more convenient for us to build the convertible cage, to develop better implant techniques, or to apply radio transmitters and tether systems. It would have been more convenient to just use the restraint chair. But we felt the need to improve conditions for the animals as the technology became available.

I think that serves as a good example of the concern people here at the primate center have for our animals. Our best protection against cruelty to animals is the people who handle the animals. I can't emphasize that enough. Our veterinarians have day-to-day contact with the animals. They care about how the animals feel. If the animal technicians think something is wrong, they report it to the veterinarians. Something is done about it immediately.

Nothing goes on here that isn't scrutinized by the veterinarians, the staff, and the scientists themselves. It's the only way. You can have all the regulations in the world, but if the people in charge of the animals don't care about them, the animals won't be treated properly. Our people care.

A lot is said about conserving research animals. Are animals at the Seattle primate center used in more than one experiment?

Oh, yes. One primate can help in several research programs. It's common to reuse animals. Our breeding station in Spokane makes it possible to conduct some experiments even before the animals are born—studies of fetal development, for example. After birth the animal may be studied in a developmental program, where observations contribute to our baseline knowledge of such factors as growth, blood characteristics, and social behavior. These animals are used in many projects.

Multiple use is a very important element of conservation. It's also very important scientifically. Many kinds of longitudinal data

are simply not available in the human population. The only place you can get these data under controlled environmental conditions is in a primate colony. We have data on a monkey that arrived as a newborn in 1957. He's 25 years old now—the equivalent of 75 years old for a human. They don't get that old in the wild. In the wild they're either quick or they're dead. When they start to slow down, their predators have them, or other group members may attack them. So, the only way to study aging is to have a colony like ours.

If an animal participates in many projects in a lifetime, would not residual effects from previous studies compromise the data?

That depends on the studies. We have records on the animals, their clinical health, and experimental history. Whenever an animal is considered for a project, the investigator gets a readout of its history. We can choose the correct animal to use for any study.

You said earlier that, at present, animals are absolutely indispensable to conducting research. The reference to time is an important qualifier. Might animals someday be replaced by alternative methods?

The use of animals will certainly continue to be reduced. I think the greatest reduction is going to come in the area of toxicity testing with the further development of tissue culture techniques. The least reduction of animal use will be in studies that require integrated organisms. Biologists need whole animals to learn how the different organs relate and interact; for example, studies in which the nervous system has to remain integrated with other parts of the body.

But you do see an increased use of alternative tests for many areas of biomedical research?

Yes, I certainly do, and I think that's good.

Do you see this change coming from external or internal forces?

It will be a combination of both. There is an increased awareness of animal welfare among the public. People are calling us to task. So, undeniably, there has been, as they say, a consciousness raising. But it's not the people outside who are making the biggest contributions. It's the people in the field who are actually making the changes. They know the problems. And, indeed, many of the solutions proposed by people outside of research are nonsense because those people don't know the problems.

One type of technology that has received a lot of attention is the computer.

That's right. Computers have achieved such an aura that people say, "Oh, you can do your experiments with the computer!" That's simply untrue. You can use the computer to analyze your data in ways that you could not before. With a computer, you may squeeze more information out of your data. But that is the full extent to which the computer can be used. You can't get anything out of a computer that you don't put into it. The computer simulation is based on data from animals, and studies of more animals are required to test the truth of predictions from the simulation. A lot of people don't recognize that.

But isn't it likely that in the future some computer simulation will be used, improved culture cell studies will be applied, and as new and better technology is developed, animals will be replaced or, at least, the number of animals needed to conduct research will be reduced?

Absolutely. Alternatives will reduce the number of animals in research. But total replacement is a long way down the line, if ever. I really doubt that we will ever be able to conduct research without any animals at all.

Microsurgery under sterile conditions at the University of Washington primate center: *adhering to detailed guidelines of analgesia, anesthesia, and postsurgical care.*

Research Focus

Therapy for Endotoxic Shock Studied in Mice

Animal experiments and data from human subjects with endotoxic shock (ES) have convinced investigators at the Medical University of South Carolina in Charleston that an inhibitor of thromboxane synthetase may improve the survival rate of patients with ES.

The drug represents a new class of pharmaceutical that inhibits synthesis of thromboxane A_2 (TXA_2), a potent vasoconstrictor and platelet aggregator, according to Dr. Perry V. Halushka, coprincipal investigator in the animal studies with Drs. James Cook and Curtis Wise. Plans are now being made to test the drug on human subjects in the late fall, says Dr. H. David Reines, the principal investigator for the proposed patient studies.

Endotoxic shock, Dr. Reines notes, is a form of septic shock caused by gram-negative bacteria. It affects the gastrointestinal tract and other parts of the body. The estimated mortality rate, investigators report, ranges from 30 percent to 70 percent, with survival depending partly on the condition of the patient before shock.

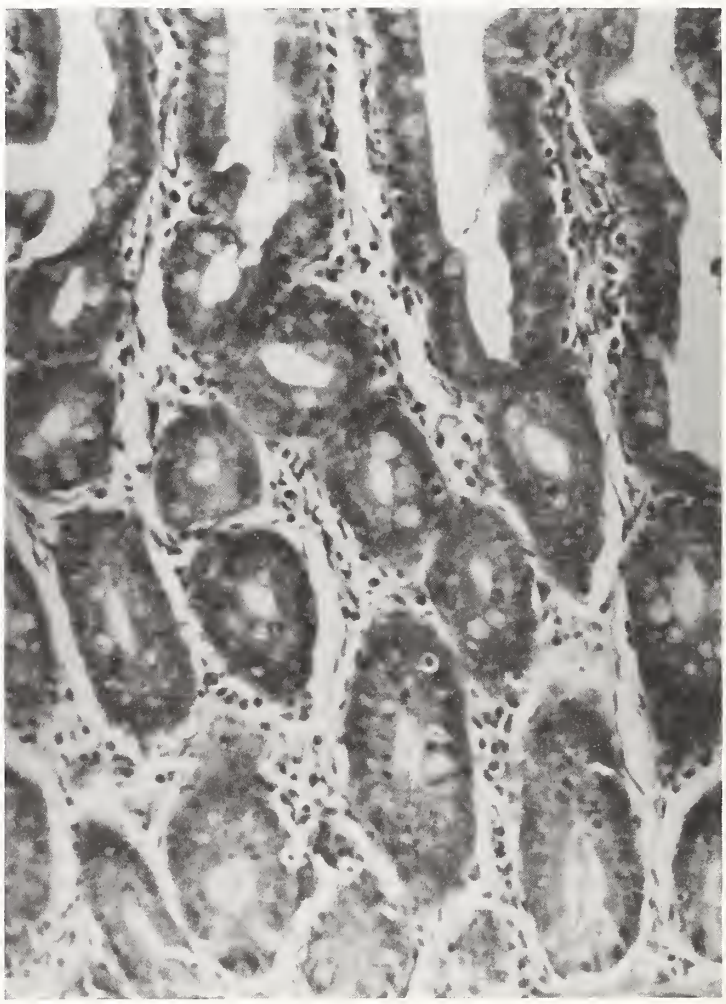
Gram-negative bacteria, which are usually present in the body without adverse effect, may infect a person who has undergone trauma or who is immunologically compromised. It is known that abdominal wounds, surgery that introduces fecal material into the peritoneal cavity, severe viral infections in children, and inflammation or obstruction of the bowel may lead to gram-negative infections. When the gram-negative bacterium dies, either naturally or because of antibiotics, it releases an endotoxin, a lipopolysaccharide, from its cell walls.

The endotoxin may cause fever, leukopenia, circulatory collapse, thrombocytopenia, disseminated intravascular coagulation (DIC), and adult respiratory distress syndrome (ARDS), which is produced by abnormally large amounts of fluid in the extracellular tissue spaces and alveoli of the lung. Animal experiments showed that ES is also associated with an increase in substances derived from arachidonic acid, a polyunsaturated fatty acid that is a component of membrane phosopholipids. This last observation put researchers on the trail that led to the possible use of the thromboxane synthetase inhibitor.

"In rats with either experimental ES or a form of septic shock, there are elevations in plasma prostaglandins and thromboxanes derived from the metabolism of arachidonic acid," Dr. Halushka says. Thromboxanes are first synthesized as the highly unstable TXA_2, which may directly cause ARDS and DIC. Oxygen supply to tissues can be reduced by ARDS, and blood from the lungs can be shunted, leading to coma and death. Researchers believe DIC may lead to the massive internal bleeding that sometimes occurs in ES.

Dr. Reines and his associates found that patients who died of septic shock had elevations of plasma thromboxane levels up to 10 times greater than did patients who subsequently survived septic shock.

To inhibit TXA_2 synthesis in laboratory animals injected with endotoxin, researchers have used thromboxane synthetase inhibitors and nonsteroidal anti-inflammatory drugs such as aspirin. Both groups of drugs have significantly prolonged the survival time of rats in endotoxic shock. The source of thromboxanes is uncertain. Drs. Halushka, Cook, and Wise believe that there are multiple potential sources, including



platelets, lungs, and reticuloendothelial cells in the liver and the spleen.

Investigators hope to use a thromboxane synthesis inhibitor in clinical tests to gain a better understanding of its potential role in human endotoxic shock. "We are hoping to find a therapy for patients with DIC and ARDS," Dr. Reines says. "If we achieve our goals, then we're hoping for improved survival."

If a successful therapy is developed from these studies, it might be used to treat victims of toxic shock syndrome, an illness mainly affecting menstruating women using tampons and diaphragms. Although toxic shock syndrome is believed to be caused by gram-positive bacteria, some of its pathological mechanisms appear to be similar to those of ES, Dr. Reines says.

Whatever the results of the proposed studies, "I don't believe we will find a 'cure all' drug for ES just like that," Dr. Reines says. "Just as there is not a single cause of cancer, there is not a single mechanism in septic shock." The use of thromboxane synthetase inhibitors may be helpful to the patient only at a particular point in the development of the syndrome. Hence, the work of investigators studying other parts of the ES puzzle also holds therapeutic promise, according to Dr. Reines. R

Additional reading:

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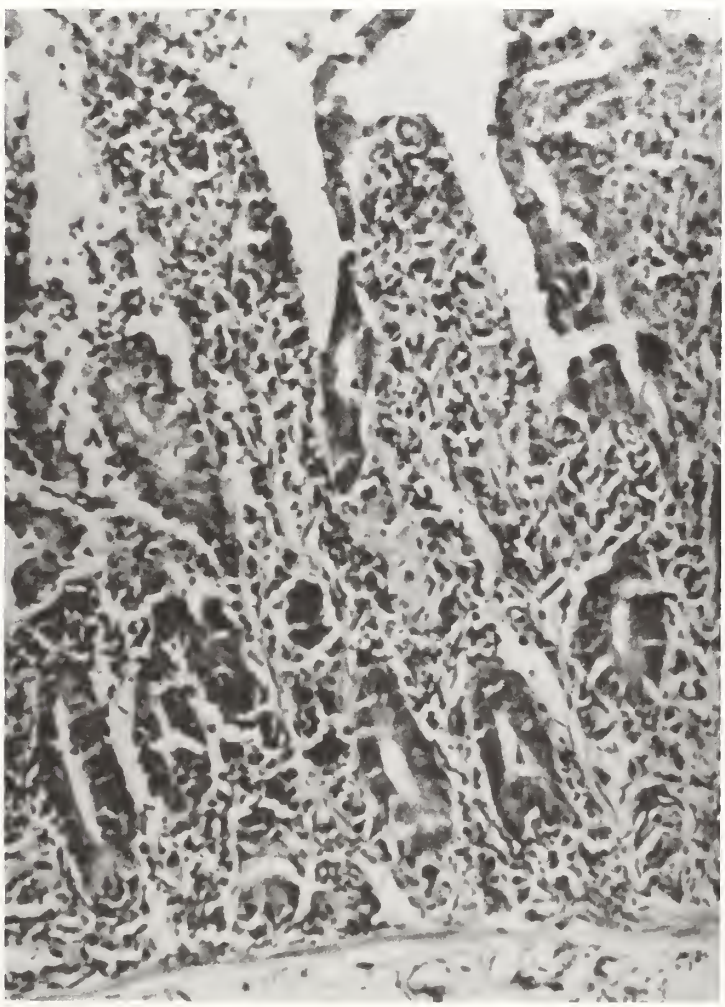
Research into endotoxic shock is supported by grants from the National Institute of General Medical Sciences, the American Heart Association, the South Carolina Heart Association, the Medical University of South Carolina Biomedical Research Appropriation, and the Biomedical Research Support Grant Program of the NIH Division of Research Resources.



Intestinal cells from rats injected with endotoxin: *cells from a rat pretreated with thromboxane inhibitor (left) appear to be normal, whereas cells from a rat not pretreated appear to be damaged.*

Research Resources Reporter
December 1982

Information Exchange

Research Highlights

Results of a study by researchers at the Duke University Medical Center in Durham, North Carolina, may contribute significantly to more accurate detection of carriers of Duchenne muscular dystrophy (DMD), a fatal genetic disease affecting male children.

Drs. Allen D. Roses, principal investigator, and Russell J. M. Lane, of the department of medicine at Duke, found a significant variation with age in serum levels of the enzyme creatine kinase (CK) in 206 unrelated normal females ranging in age from 1 to 70. The importance of these data is underlined by the fact that the CK test is presently regarded as the best test and is the test most widely used for detection of DMD carriers. The CK level in serum is higher than normal in a large percentage of definite carriers of the disease. Detection of carriers, followed by genetic counseling, is at present the only way to control DMD.

Duchenne muscular dystrophy, named after the French physician who first described the disorder in 1861, is transmitted by mother to son. Only males develop DMD. Symptoms usually become apparent between the ages of 2 and 6. DMD is characterized by a gradual wasting away of muscle, accompanied by weakness and crippling. The disease steadily becomes worse, and children usually do not survive beyond adolescence. The cause of DMD is unknown and there is no specific therapy.

Each son of a mother who carries the Duchenne trait has a 50 percent risk of inheriting the disease, and each of her daughters has a 50 percent risk of being a carrier. Carriers very rarely have any overt signs of DMD. There are approximately 10,000 children with DMD now living in the United States, according to the Muscular Dystrophy Association, which estimates that the risk of the disease is about 1 in 4,000 live male births.

In the study conducted by Drs. Roses and Lane, CK levels were highest during the first few months of life, fell rapidly until about age 4, increased to a peak around 10 to 12 years of age, and then fell abruptly around commencement of the menstrual cycle. CK levels continued to decline slowly throughout adult life until about age 40, when there was a slight rise.

The purpose of the study, according to Dr. Roses, was to establish normal CK ranges for females at different ages—that is, age-specific CK ranges.

The fluctuation of CK values with age should be taken into account, Dr. Roses indicates, when the CK test is used for detecting DMD carriers. If the normal CK value used as the control is lower than the CK range for the age of the person being tested, normal individuals could be incorrectly classified as carriers on the basis of the CK test (false positives). On the other hand, if the control CK value used is higher than the CK range for the subject's age, carriers could be missed (false negatives). In practice, however, CK levels are incorporated with genetic data from pedigrees, for counseling purposes.

Dr. Roses states that the CK test may be most discriminating for carrier detection in children at ages 3 to 5, when the CK level in normal girls is at its lowest point.

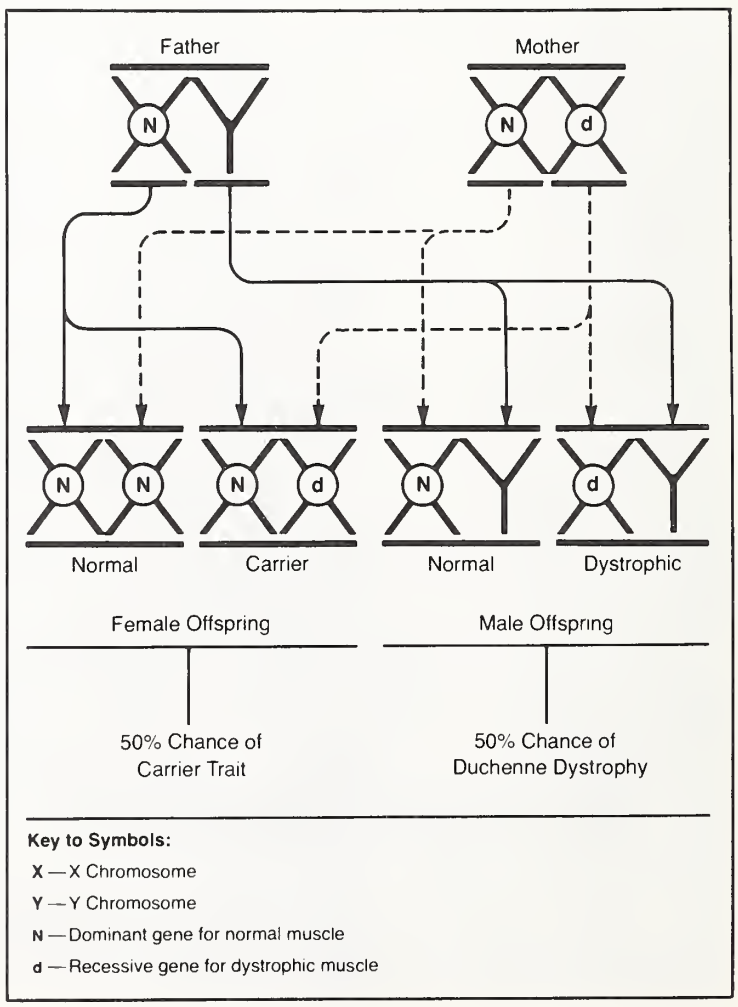
Research has indicated that CK levels are higher than normal in about 70 percent of *definite carriers* and of *probable carriers* and in about 50 percent of *possible carriers* of Duchenne muscular dystrophy.

Definite carriers are mothers who have a son with DMD and a previous case of DMD on the maternal side of the family. *Probable carriers* are mothers of two or more sons with the disease and have no other affected relatives. *Possible carriers* are mothers of a single isolated case or are a relative of an affected male.

Dr. Roses points out that the CK test is a nonspecific test since CK levels can be elevated in a number of other diseases. Some of these diseases can be ruled out by the sex of the patient, since clinical DMD occurs only in males. "Other diseases can usually be ruled out by the case history, the physical examination, and the age and sex of the patient," Dr. Roses says. "On the other hand, there are several diseases that require muscle biopsy for differential diagnosis of DMD. Biopsy is performed in all cases for which there is no family history."

Detection of DMD carriers will be more accurate when the biochemical defect in the disorder is known and potential carriers can be tested specifically for that defect, according to Dr. Roses. "Until that time, use of nonspecific measurements such as CK requires careful laboratory controls and integration of data into useful models for genetic counseling," he says.

Other procedures that have been suggested as possible DMD carrier detection tests include muscle biopsy, electrocardiography, and electro-



myography. Research has demonstrated microscopic abnormalities in muscle biopsy specimens from *definite carriers,* but the procedure has been recommended only for those medical centers that have the necessary expertise in taking satisfactory biopsy specimens and have data on a large series of known carriers and normal persons as a basis for interpreting test results.

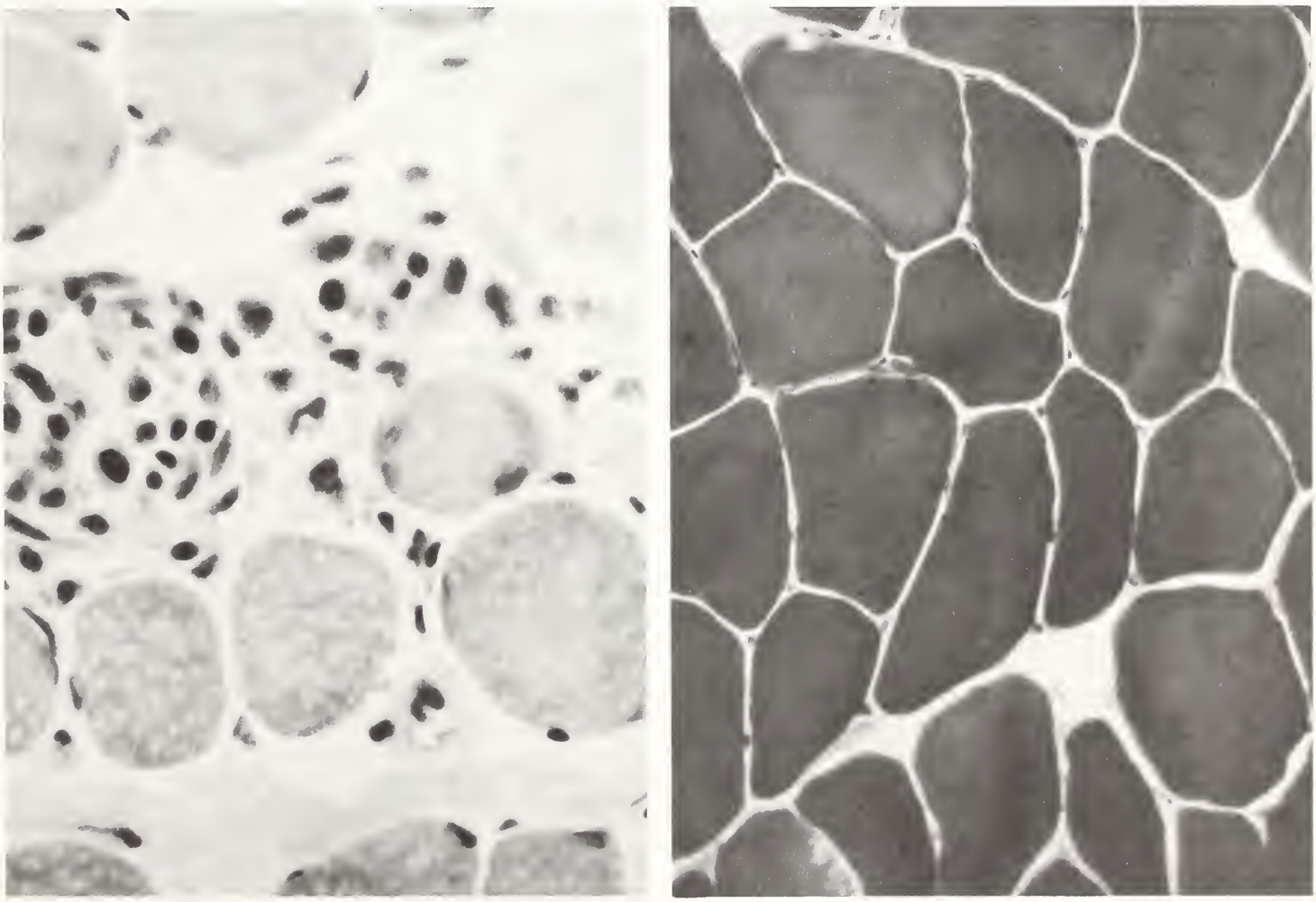
Definite carriers with normal CK levels have been detected by electromyography, which records electrical patterns of skeletal muscle at rest and during contraction. Abnormalities in ECG have also been found in DMD carriers.

"Although the CK test is regarded as the best to date for detection of DMD carriers, it has a lot of drawbacks," Dr. Roses states. Subjective factors that affect the CK level include age, exercise, pregnancy, and individual variation from time to time in carriers. Some drugs also influence CK levels, but oral contraceptives have no significant effect. Inaccurate CK test values may also result from mishandling specimens and the use of unreliable analytical methods. To improve the value of the CK test, all of these factors must be taken into consideration.

Strenuous exercise should be avoided for 1 week prior to the test since it increases CK levels in both carriers and normal persons. Individuals vary considerably, however, in their response.

"Pregnancy almost always causes a decrease in the CK level," Dr. Roses says. Consequently, it is important to complete carrier testing and genet-

Loss of muscle cells and replacement by connective tissue in Duchenne muscular dystrophy (left) compared to normal muscle cells (right): *a crippling disorder characterized by a gradual wasting away of muscle.*



ic counseling before a pregnancy occurs.

CK levels vary considerably from time to time in some carriers. Therefore, it has been recommended that at least three tests be performed at intervals greater than 1 week. Dr. Roses emphasizes the importance of using the mean value of several samples for genetic counseling in the case of potential carriers with high or borderline CK levels.

Serum specimens should be protected from bright light and temperature fluctuations. Delay in analysis may also invalidate CK test results if the specimen is not preserved properly. In the study by Drs. Roses and Lane, to ensure accuracy, serum specimens were analyzed immediately or after storage at minus 20 degrees centigrade for a maximum of 2 weeks.

The analytical procedure used must yield both accurate and reproducible results. The importance of this is underlined by recent research that revealed marked differences in results on identical samples analyzed in different laboratories.

New research designed to find better methods of carrier detection has focused mainly on identification of the basic defect in the disease, location of the abnormal gene on the chromosome, and the abnormality in the gene itself.

Recent work suggests that there may be a generalized membrane defect in patients with DMD and that the expression of the abnormal gene is not confined to muscle. The fundamental defect could be in another organ, and the muscle abnormalities may be secondary. The actual cause of the disease beyond the genetic defect has eluded researchers so far.

Two main types of techniques are being used to locate the area on the chromosome where the genetic defect resides. One approach, mapping the DMD gene, may reveal neighboring marker systems whose inheritance may aid in the detection of DMD carriers. An example of the value of this approach is the discovery that the location of the gene for Becker muscular dystrophy is linked to that for a type of color blindness. This technique is based on the principle that each gene has a particular and well-defined location on its chromosome and that neighboring genes tend to remain together.

Dr. Roses and his colleagues at Duke are currently employing a different approach. They are using recombinant DNA techniques to search for genetic markers close to the Duchenne trait on the X chromosome. Recombinant DNA techniques may also lead to identification of the defect in the DNA of the gene itself. A probe in the area of the DMD locus might provide the basis for a direct and specific test for carriers, since practically all readily available cells of the body could be used for the test.

The Duke GCRC was used for examining some of the patients and normal controls enrolled in the study and also for obtaining some of the specimens for the CK tests, Dr. Roses says. Research by the Duke Neuromuscular Research Clinic has been centered in the GCRC since 1974.

Additional reading:

1. Lane, R. J. M., and Roses, A. D., Variation of serum creatine kinase levels with age in normal females: implications for genetic counseling in Duchenne muscular dystrophy. *Clinical Chimica Acta* 113:75–86, 1981.
2. Harper, P. S., Carrier detection in Duchenne muscular dystrophy: a critical assessment. In *Disorders of the Motor Unit* (Schotland, D. L., ed.). New York, N.Y., John Wiley & Sons, 1982, pp. 821–846.
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7. Siegel, I. M., *101 Questions & Answers About Muscular Dystrophy*. New York, N.Y., Muscular Dystrophy Association, 1981, pp. 1–21.
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Research into variation of serum creatine kinase levels with age in normal females was supported by the National Institute of Neurological and Communicative Disorders and Stroke and the Muscular Dystrophy Association. The GCRC at Duke University Medical Center is supported by the NIH Division of Research Resources. Dr. Lane is now at the Muscular Dystrophy Group Research Laboratories, Newcastle General Hospital, Newcastle upon Tyne, United Kingdom.

Research Service

DNA–RNA Data Bank

An international data bank of DNA and RNA sequences is now available. The data bank, called GenBank, enables scientists to obtain the sequences of DNA and RNA molecules comprised of approximately 750,000 bases.

Information in the data bank is being gathered, annotated, and organized by Dr. Walter Goad and his staff at the Los Alamos National Laboratory in New Mexico. A scientific research firm, Bolt, Beranek, and Newman, Inc. (BBN), Cambridge, Massachusetts, maintains the computerized data and distributes the information to subscribing institutions and scientists via magnetic tape and limited online computer access by telephone. One means of access is the PROPHET computer system, which was developed and is being maintained by the Biotechnology Resources Program of the NIH Division of Research Resources (DRR). A compendium of data in GenBank will be published annually.

An effort to develop the nucleic acid data base began at Los Alamos 3 years ago. The Los Alamos data bank and other genetic-sequence-handling tools have been available on the PROPHET system for several years. These efforts resulted in the current project, which is being funded by the National Institute of General Medical Sciences through a contract to BBN. Other agencies funding GenBank through the same contract include the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the DRR Biotechnology Resources Program, the National Science Foundation, the Department of Energy, and the Department of Defense.

The purpose of the data bank is to avoid the duplication of data collection that might occur in a rapidly expanding

field such as molecular genetics. The speed at which new knowledge is gained makes it difficult for scientists to organize and interpret the information. More than 500,000 nucleic acids are sequenced each year. Understanding sequence patterns enables scientists to recognize structural features and coding relationships within DNA molecules that correspond to specific biological functions within a cell. Research in this field may ultimately help scientists understand the processes of disease as well as inheritance.

Sequences of the nucleic acids in DNA and RNA are ordered lists of four types of smaller molecules called bases. Nucleic acid molecules range in size from 100 bases to hundreds of millions of bases. The sequence of Epstein-Barr virus, which contains 170,000 bases, is expected to be published within the year.

According to Dr. Goad, the library will be brought up to date within the year. Thereafter, sequences will be collected within 3 months of their publication. The Los Alamos investigators will use information from more than 20 journals and will develop international collaborations to compile a definitive bank of DNA and RNA sequences.

Scientists who are interested in gaining access to the DNA–RNA data bank can obtain additional information by contacting:

Dr. Howard Bilofsky
GenBank
c/o Computer Systems Division
Bolt, Beranek, and Newman, Inc.
10 Moulton Street
Cambridge, Massachusetts 02238
(617) 497-2742

Meetings

Computers in Medicine Congress Announced

Computer applications in medicine will be the theme of the 2nd Annual Joint National Congress of the American Association for Medical Systems and Informatics (AAMSI) CONGRESS 83.

The conference, which will be held in San Francisco, California, from May 2 to 4, 1983, will provide a forum for physicians, nurses, health care administrators, allied health professionals, computer scientists, and bioengineers. Topics to be discussed include body imaging, X-ray and ultrasound technology, computer networks, artificial intelligence, nuclear medicine, and robotics. Ambulatory care and office practice, clinical decision-making, emergency systems, and biomedical research also will be addressed. Panel discussion will focus on clinical laboratory systems, and computer-aided medical and health education. For further information and for an application to submit papers, contact:
Dr. M. F. Collen
3451 Piedmont Avenue
Oakland, California 94611
(415) 428-6688

Conferences in Biochemistry

The annual Gordon Research Conferences, sponsored by the National Science Foundation, will be held January 10 to February 25, 1983, in Ventura, California, and January 10 to February 4, 1983, in Santa Barbara, California. The 14 conferences will be held in two cities to provide hotel accommodations for guest speakers and the approximately 1,800 invited attendees. Ten conferences will be held in Ventura, and four will be held in Santa Barbara. Each conference will last 4 to 5 days.

The conferences will update experts on the latest research developments in chemistry and related sciences. Researchers attending the conferences will discuss and analyze the significance of research developments and their underlying theories. Approaches to conducting research in these areas will be addressed.

Conferences to be held in Ventura will be on such topics as immunochemistry and immunobiology, prolactin, agricultural science, and the role of oxygen radicals in biology and medicine. Conferences to be held in Santa Barbara will be on topics including polymers, electrochemistry, and the chemistry and physics of isotopes.
For additional information, contact:
Dr. Alexander M. Cruickshank
Director
Gordon Research Conferences
University of Rhode Island
Kingston, Rhode Island 02881
Telephone (401) 783-4011

January 12–14, 1983

Title: *The Chemistry and Biology of Alpha–2 –Macroglobulin*
Location: *New York , New York*
Contact: *Conference Director*
The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021
Telephone (212) 838-0230

January 17–21, 1983

Title: *Advances in Gene Technology: Molecular Genetics of Plants and Animals*
Location: *Miami Beach, Florida*
Contact: *Miami Winter Symposium*
P.O. Box 016129
Miami, Florida 33101
Telephone (305) 547-6265

January 22–29, 1983

Title: *2nd Winter Congress of the International Body Imaging Conference*
Location: *St. Moritz, Switzerland*
Contact: *Secretary*
Winter Congress
West Park Hospital
22141 Roscoe Boulevard
Department of Radiology
Canoga Park, California 91304
Telephone (213) 340-0580, ext. 280

February 12–19, 1983

Title: *2nd Winter Symposium on Hematologic Malignancies*
Location: *Snowbird, Utah*
Contact: *S. E. Jones, M.D.*
Section of Hematology and Medical Oncology
University of Arizona Cancer Center
Tucson, Arizona 85724
Telephone (602) 626-6372

February 19–24, 1983

Title: *4th International Workshop on Neurological Surgery of the Ear and Skull Base*
Location: *Sarasota, Florida*
Contact: *H. Silverstein, M.D.*
Ear Research Foundation
1921 Floyd Street
Sarasota, Florida 33579
Telephone (813) 365-0367

March 6–11, 1983

Title: *83rd Annual Meeting of the American Society for Microbiology*
Location: *New Orleans, Louisiana*
Contact: *Richard A. Bray*
1913 I Street, N.W.
Washington, D.C. 20006
Telephone (202) 833-9680

March 10–15, 1983

Title: *American Academy of Orthopedic Surgeons' 50th Anniversary Meeting*
Location: *Anaheim, California*
Contact: *C. V. Heck, M.D.*
American Academy of Orthopedic Surgeons
444 North Michigan Avenue
Suite 1500
Chicago, Illinois 60611
Telephone (312) 822-0970

March 13–17, 1983

Title: *9th Annual Meeting of the Clinical Ligand Assay Society*
Location: *Philadelphia, Pennsylvania*
Contact: *Katherine Boyle*
Wayne County General Hospital
P.O. Box 67
Wayne, Michigan 48184
Telephone (313) 722-6290

March 17–20, 1983

Title: *Annual Session of the American Association for Dental Research*
Location: *Cincinnati, Ohio*
Eloise Ullman
Contact: *AADR*
734 15th Street, N.W.
Suite 809
Washington, D.C. 20005
Telephone (202) 638-1515

March 19–23, 1983

Title: *39th Annual Meeting of the American Academy of Allergy and Immunology*
Location: *Hollywood, Florida*
Contact: *D. L. McNeil*
611 East Wells Street
Milwaukee, Wisconsin 53202
Telephone (414) 272-6071

March 20–25, 1983

Title: *185th National American Chemical Society Meeting*
Location: *Seattle, Washington*
Contact: *Paula Groves*
1155 16th Street, N.W.
Washington, D.C. 20036
Telephone (202) 872-8072

April 11–15, 1983

Title: *1st World Congress on Cardiovascular Pharmacotherapy*
Location: *Geneva, Switzerland*
Contact: *Dr. Adam Schneeweiss*
12 Avenue des Amazones
1224 Chene-Bourgeries
Geneva, Switzerland

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